Relationship between microalgae lipid extracts composition and rheological properties

Mariane Audo, Emmanuel Chailleux, B Bujoli, C Queffelec, J Legrand, O Lepine

Relationship between microalgae lipid extracts composition and rheological properties

Audo M., Chailleux E., Institut Français des Sciences et Technologies du Transport, de l'Aménagement et des Réseaux (IFSTTAR) Route de Bouaye, 44340 Bouguenais, +33(0)240845986, mariane.audo@ifsttar.fr

Bujoli B., Queffélec C., Chimie et Interdisciplinarité : Synthèse, Analyse, Modélisation (CEISAM) 2 Rue de la Houssinière, BP 92208, 44322 Nantes Cedex 03.

Legrand J., Université de Nantes, CNRS, GEPEA, UMR 6144, 37, bd de l'Université, BP 406 44602 Saint-Nazaire Cedex (France)

Lépine O., Alpha Biotech, Le Frostidié, 44410 Assérac

Abstract: Renewable energy sources are developed worldwide due to high oil prices and to limit greenhouse gas emissions. In this context, some groups have focused their work on vegetable oils, and particularly, on microalgae. The last decade has seen an increasing scientific interest in the extraction of lipids from microalgae for the production of biodiesel. Microalgae present main advantages, compared to other energy crops including a high growth rate, a high biomass production, and do not compete with human feeding production. For economical and ecological reasons, the by-products resulting from the microalgae culture have to be valorised. This is what we propose to do in the "Algoroute" project, trying to understand if it could be used as a binder for aggregates. To achieve this goal, we need to figure out the composition of those by-products, and see the influence of each class of compounds on the rheological properties. Extraction and transformation steps have also to be studied taking into consideration low energy and environmentally friendly processes. Interesting preliminary results have been obtained. Several fractions from micro-algae have been extracted and characterized by IR, NMR (^{1}H and ^{13}C) and GC-FID. The complex modulus was also determined on these different fractions. The comparison of rheological and chemical analyses allows to highlight some chemical species which show a thermo-dependent behaviour comparable to asphalt.

1. Introduction

The last decades have seen a growing interest in renewable energies, due to the rarefaction of the petroleum resources, high oil prices, and to limit greenhouse gas emissions. First and second generation biofuels (based on sugar, starch or vegetable oil for the first one, and on lignocellulosic biomass for the second one) might be challenged by third generation biofuels based on microalgae. Microalgae are one of the most promising alternatives in this field, as they present main advantages compared to other terrestrial plants, which lead to a high productivity (Figure 1):

- high biomass production ;
- high growth rate ;
- high photosynthetic yield ;
- high lipid content (until 80 weight% under stressing conditions and for some particular species)
- no competition with human feeding.

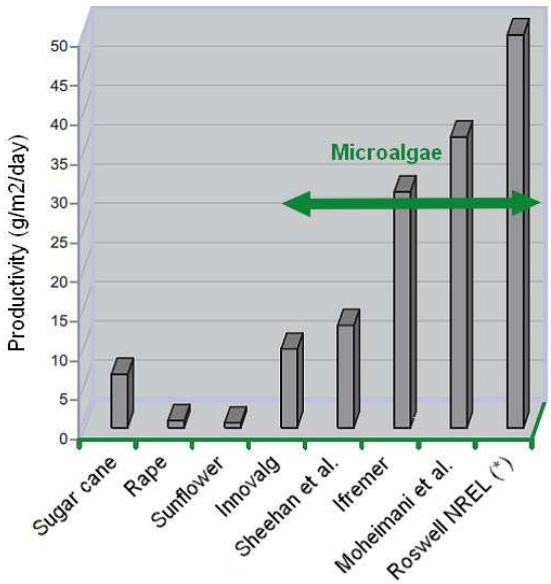

Figure 1: Oil productivity of several biomasses (adapted from [1])

Microalgae are also an extraordinary source for feeding, chemicals or biomedical molecules [2]. But the microalgae industry can be viable only if all fractions of biomass can be valorised, as for petroleum. That is why we propose to explore the possibility to develop an alternative binder based on the valorisation of microalgae by-products.

Many alternative binders, derived from biomass, are already developed by some firms like Colas, Shell... They are principally based on a mixture between a vegetable oil (linseed, rape…) and a chemically modified pine resin, which allows to give a higher viscosity to the blend [3].

Consequently, using by-products of microalgae represents an original approach as no edible oil will be used and their will be no competition with other uses of biomass (cosmetic, biofuels, molecules for chemistry…).

Here, we propose to use some residues of a microalga from which some proteins have been removed for another application. Consequently, our raw material is rich in lipid and carbohydrates. The precise chemical composition has to be known, in order to see the influence of each class of compounds on the rheological properties. In this paper, we will focus our attention on the study of the lipid part valorisation.

2. Materiel and methods

2.1 Microalgae preparation

Microalgae were obtained from Alpha Biotech, in Assérac (France). The major part of water-soluble proteins was removed by centrifugation. Residue of microalgae, containing about 80% of water, was frozen, and then freeze-dried for one week at -90 °C.

2.2 Extraction and fractioning methods

A Soxhlet apparatus was used to extract lipids from microalgae. About 4 g of microalgae powder was loaded in a 70 mL cellulose thimber. 90 mL of n-hexane, chloroform, and

chloroform/methanol (2:1, v/v) were successively used, for 24 h. Solvents were removed under vacuum, at 40 °C. Oil yields were determined by gravimetry.

A sonication of the sample in CCl_4 allowed us to fractionate the oil in two parts: a CCl_4 soluble fraction and a CCl_4 insoluble one, which were separated by simple filtration.

2.3 Fatty acid methylation

Fatty acid methyl esters (FAMEs) were prepared according to [4]. 12 mL of boron trifluoride in methanol (14%) were added to 200 mg of the lipid extract. The mixture was heated in a sealed tube at 90 °C overnight. FAMEs were extracted by hexane, washed with water and dried over $MgSO_4$. The solvent was removed under vacuum at 40 °C and FAMEs were dissolved in isopropanol for their analysis by gas chromatography.

2.4 Analytical methods

Nuclear Magnetic Resonance (NMR): Samples were dissolved in a deuterated solvent (*e.g* $CDCl_3$, MeOD or D_2O). ^{1}H NMR spectra were recorded on a Bruker AVANCE (DPX 300 or 400) Ultrashieldand. ^{13}C NMR spectra were recorded at 75 MHz or 100.6 MHz. ^{31}P spectra were recorded at 121 MHz or at 161.97 MHz.

Gas-chromatography: Gas-chromatography was performed on a Agilent 6890 equipped with a flame ionization detector and a BPX70 capillary column (70% cyanopropyl dimethylpolysiloxane, 30 m x 0.32 mm ID, 0.25 µm film thickness). Helium was used as carrier gas at 1.3 mL/min. The oven temperature was 120 °C, held for 4 min, raised to 220 °C at a 6 °C/min rate and held at 220 °C for 5 min. It was then raised to 250 °C at a 15 °C/min rate, and held at 250 °C for 18 min. The injector and detector temperature were set at 250 °C. The identification of fatty acids was performed by comparison with the retention time of standards.

Infrared: IR spectroscopy was carried out with an IR-TF Vector 22. Oil samples were dissolved in CCl_4 and placed into a KBr window for the acquisition. A KBr pellet was used for the analysis of solid samples (1% in weight).

Differential Scanning Calorimetry: DSC curves were obtained with a Netzsch DSC 200 device. Measurements were performed from -75 °C to 125 °C, in aluminium crucibles.

Rheology: The complex modulus and phase angle of the samples were measured with a Metravib viscoanalysor DMA+450, in the shear mode, at a 1 Hz to 125 Hz frequency range, and 10 °C to 80 °C temperature range.

3. Results and discussions

3.1 Soxhlet extracts composition

Three successive extractions were performed in order to fractionate lipids according to their polarity and chemical structure. For hexane, chloroform, and chloroform/methanol, the extraction yields are 5%, 5% and 12% respectively, which lead to a 22% cumulative yield.

Further sonication in CCl_4 showed the existence of an insoluble fraction (0% in hexane extract, 15% in chloroform extract, and 35% in chloroform/methanol extract).

A NMR study showed a similarity between the spectra of the three CCl_4 soluble extracts (Figure 2 and Table 1). The extracts are only composed of free fatty acids. Even though there is no physiological reason for microalgae to produce free fatty acids, they were the only lipids

observed by liquid ^{1}H and ^{13}C NMR. This could be the result of an enzymatic lysis of more complex lipids during the preliminary protein extraction process.

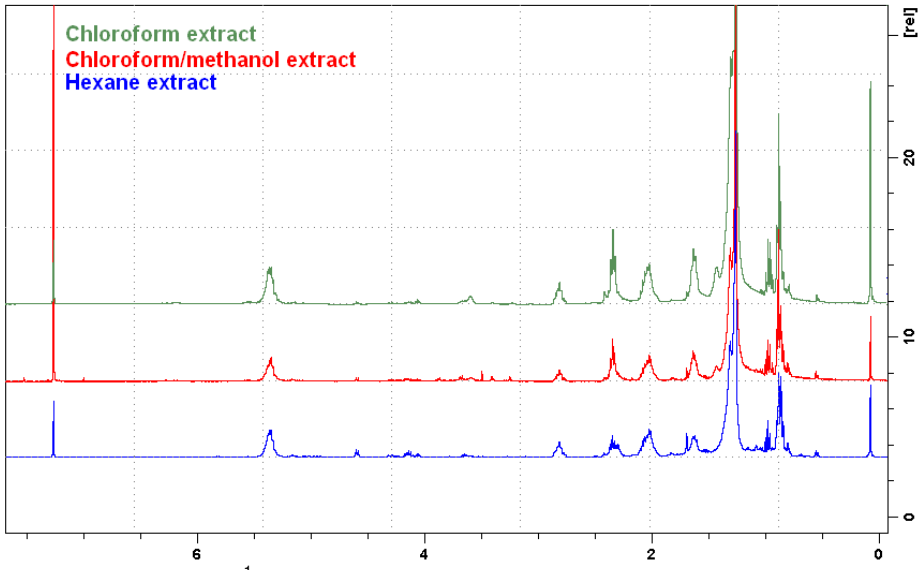

Figure 2: ^{1}H NMR spectrum comparison of the three extracts

Table 1 : ^{1}H NMR assignment

δ (ppm)	Chemical structure
0.9	$C\underline{H}_3$-$(CH_2)_n$- of ω6 and ω9
1	$C\underline{H}_3$-$(CH_2)_n$ of ω3
1.2	-$(CH_2)_n$-
1.6	-$C\underline{H}_2$-CH_2-COO-
2.0	-$\underline{H}_2$C-HC=CH-$C\underline{H}_2$-
2.3	-H_2C-COO-
2.8	=HC-$C\underline{H}_2$-HC=
5.3	-HC=CH-

To allow their analysis by gas chromatography, free fatty acids were analyzed as their methyl esters. A total of 18 fatty acids were identified, the major ones being palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid as seen in Figure 3 (classical ones found in microalgae). Table 1 shows the assignment of each peak, based on a comparison with free fatty acids references.

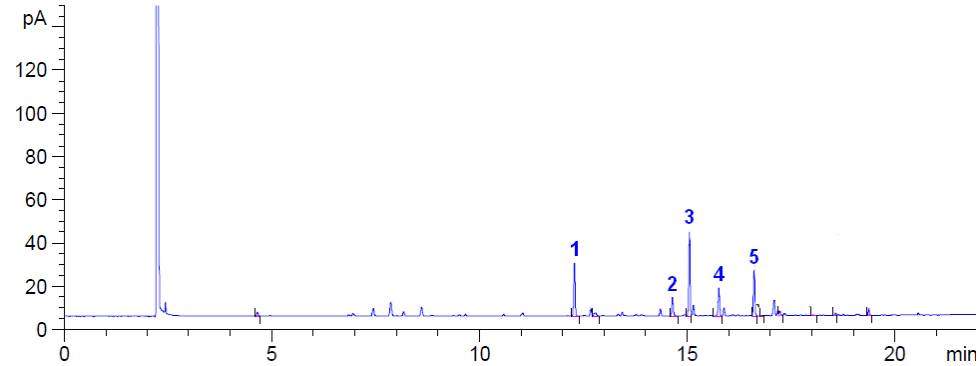

Figure 3: Typical GC profil of FAMEs obtained by Soxhlet extraction (1=C16:0; 2=C18:0; 3=C18:1; 4=C18:2; 5=C18:3)

Other analytical techniques have to be used to analyse the CCl_4 insoluble extract which is insoluble in every organic solvent, thus making the liquid NMR method unsuitable.

The infrared spectrum of the CCl_4 insoluble extract (Figure 4) shows some specific absorption bands. The broad band around 3400 cm^{-1} is attributed to the O-H elongation of some hydroxyl groups. Both signals at 2915 and 2850 cm^{-1} are due to the C-H elongations of CH_2 and CH_3. The broad band centre around 1640 cm^{-1} can be attributed to the C=O elongation, which suggests the presence of polyester. From comparison with literature data [5], we suspect the CCl_4 insoluble extract to be algaenans, a highly resistant and non-hydrolysable biopolymer, already encountered in some microalgae [6], [7]. Further analyses have still to be done to confirm this hypothesis.

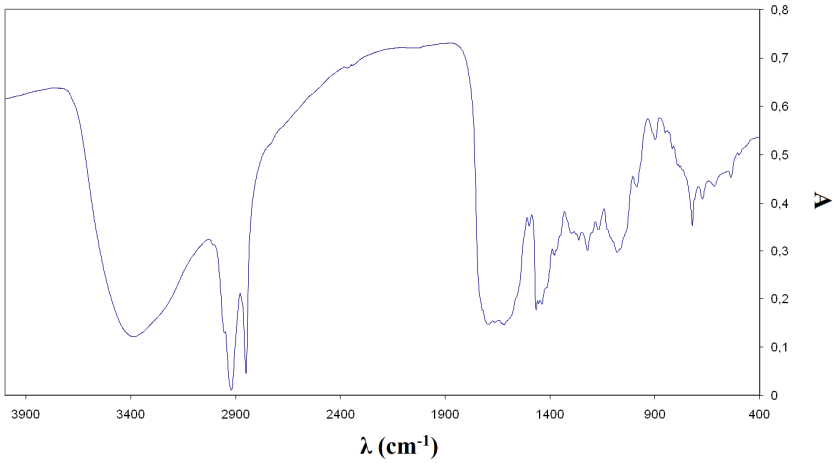

Figure 4: IR spectrum (KBr pellet) of CCl_4 insoluble extract

3.2 Properties of Soxhlet extracts

In order to have a better understanding of the influence of each constituent (*i.e.* free fatty acids and algaenans) on the physical properties of the mixtures, we performed some DSC (Figure 5) and rheological studies (Figure 6) while varying the algaenans content.

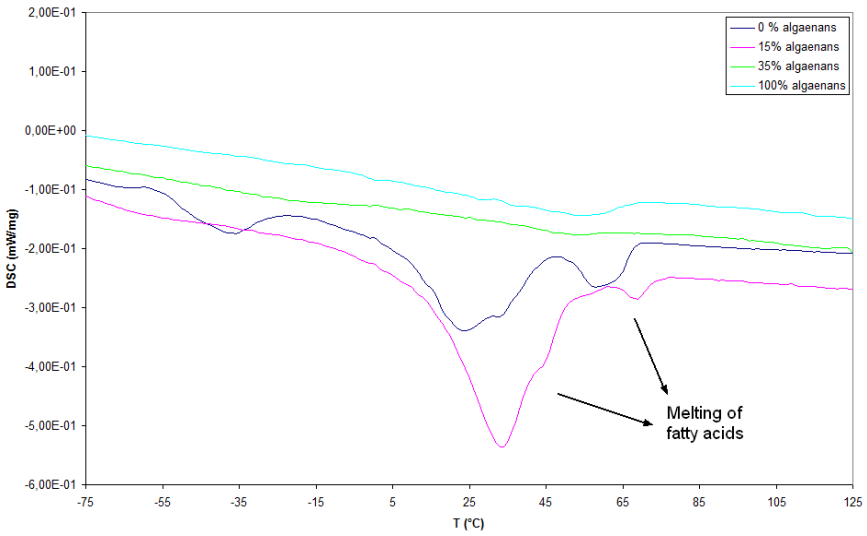

Figure 5: Evolution of DSC curves according to the algaenans content

The behaviour of the fractions containing either 0% or 15% of algaenans is dominated by the melting of free fatty acids between 15 °C and 80 °C (the shift between both curves may be attributed to a variation of length and degree of unsaturation of the fatty acids). An increase of the algaenans content leads to the disappearance of the thermal transitions due to fatty acids. The only visible transition (around 60 °C) is the one which is associated to algaenans, as it is also detected for the pure sample of algaenans.

Rheological properties (phase angle and norm of complex modulus) were also measured on three samples containing 0%, 20% and 35% of algaenans. An easy way to visualize the differences between all the samples is to draw the Black curves (plotting phase angle φ against the norm of complex modulus |E*|).

As seen in Figure 6, the sample composed by only free fatty acids shows a discontinuity between all the isotherms. This is related to a molecular reorganization at each temperature (*i.e* melting of free fatty acids). An increase from 0% to 20% of the algaenans content results in a better arrangement of the isotherms, although still discontinuous. A totally different behaviour is observed at 35% of algaenans. In this case, as for bitumen, there is a continuity between each isotherm, showing the thermo-rheological stability of the material which appears to behave as an usual bituminous binder.

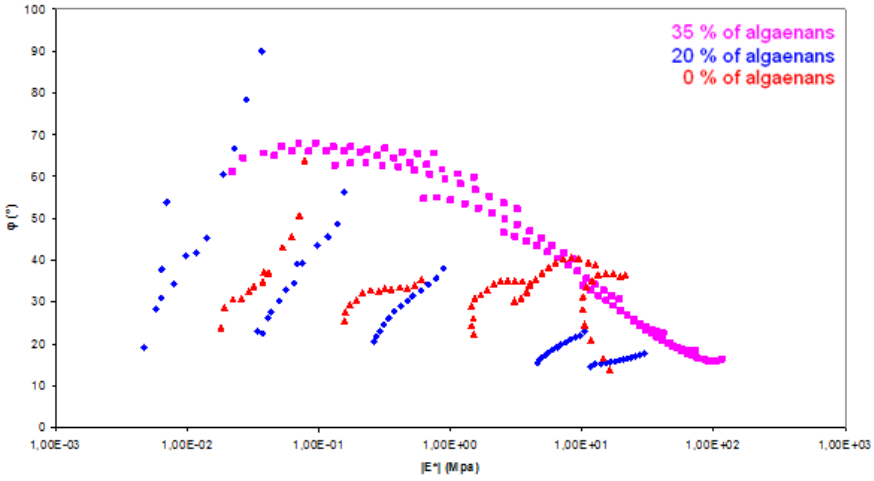

Figure 6 : Variation of Black curves as a function of the algaenans content

The complex modulus norm of microalgae extracts, measured at 1 Hz, is compared to two paving grade asphalts. On the temperature range tested, microalgae Soxhlet extracts show a thermosusceptibility similar to the one of asphalt. Moreover, it seems possible to adjust the rheological properties of the material by tuning the algaenans content. In this way, it might be possible to obtain alternative road binders with the same rheological properties as asphalts.

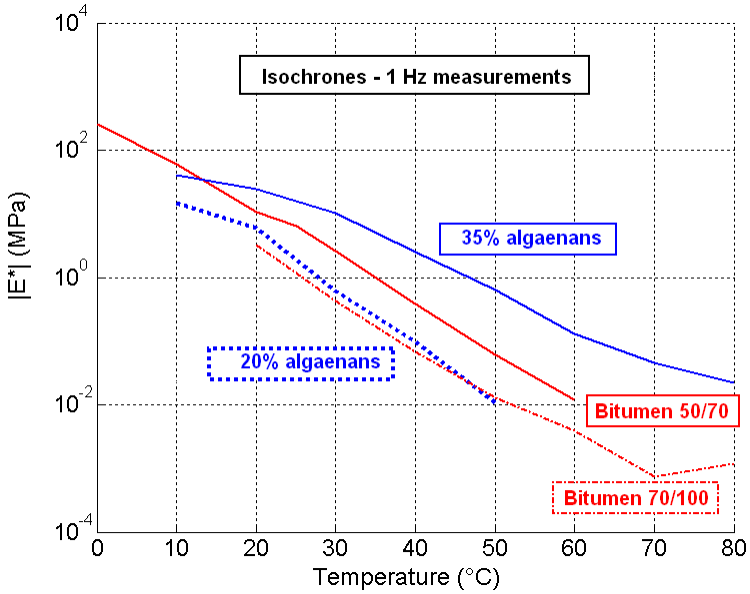

Figure 7 : Rheological comparison between microalgae extracts and bitumen

Conclusions

Through this study, we suggest that the design of microalgae-based road binders should be feasible. The isolated lipid fraction of the studied microalgae is made of a fatty acid polymer (algaenans) suspended in a free fatty acids oil (principally palmitic, stearic, oleic, linoleic and linolenic acids). This thermo fusible viscoelastic material shows rheological properties similar to those of asphalt. These rheological properties can be tuned by varying the algaenans content in the mixture. It could be a way to provide to a wide range of bitumens.

While only a fraction of the microalgae residue was addressed in the present paper, we now need to investigate innovative and environmentally clean processes (*i.e* low energy-demanding and solvent-free protocols) that would allow full valorisation of the total residue.

In a subsequent stage, it will be necessary to evaluate the mechanical properties of this material, and its potential durability.

References

[1] J.-P. Cadoret, O. Bernard, La production de biocarburant lipidique avec des microalgues : promesses et défis, *J. Soc. Biol.* **202** (3) (2008) 201–211.

[2] C. Vilchez, I. Garbayo, M. V. Lobato, J. M. Vega, Microalgae-mediated chemicals production and wastes removal, *Enzyme Microb. Technol.* **20** (8) (1997) 562–572.

[3] Ballie, M., Liant de nature végétale pour la réalisation de matériaux pour le bâtiment et/ou les travaux publics Patent FR 2853647. October 15, 2004

[4] W. R. Morrison, L. M. Smith, Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride methanol, *J. Lipid Res.* **5** (4) (1964) 600–608.

[5] P. Blokker, S. Schouten, H. van den Ende, J. W. de Leeuw, P. G. Hatcher, J. S. S. Damsté, Chemical structure of algaenans from the fresh water algae *Tetraedron minimum*, *Scenedesmus communis* and *Pediastrum boryanum*, *Org. Geochem.* **29** (5-7) (1998) 1453–1468.

[6] B. Allard, J. Templier, High molecular weight lipids from the trilaminar outer wall (tls)-containing microalgae *Chlorella emersonii*, *Scenedesmus communis* and *Tetraedron minimum*, *Phytochemistry* **57** (3) (2001) 459–467.

[7] B. Allard, M.-N. Rager, J. Templier, Occurrence of high molecular weight lipids (c80+) in the trilaminar outer cell walls of some freshwater microalgae. a reappraisal of algaenan structure, *Org. Geochem.* **33** (7) (2002) 789–801.